Data Evaluation Report on the acute toxicity of Ethylene urea (Degradate of ETU) on the Freshwater Green Alga, *Selenastrum capricornutum*

PMRA Submission #:{.....} EPA MRID #: 464629-04

Data Requirement::	PMRA DATA CODE	{.....}
	EPA DP Barcode	D305127
	OECD Data Point	{.....}
	EPA MRID	464629-04
	EPA Guideline	850.4400 (previously FIFRA 123-2)

Test material: Ethylene urea (EU) **Purity:** 90.8%
Common name: Degradate of Ethylenethiourea (ETU)
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No.: Not reported
Synonyms: BAS 222 F

Primary Reviewer: Gregory Hess **Signature:**
Staff Scientist, Dynamac Corporation **Date:** 4/11/04

QC Reviewer: John Marton **Signature:**
Staff Scientist, Dynamac Corporation **Date:** 4/14/04

Primary Reviewer: Brian Montague, Fishery Biologist **Signature:**
OPP/EFED/ERB-V **Date:** February ,2015

Secondary Reviewer: Allen W. Vaughan **Date:** 04/16/15
PMRA

Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
EPA PC Code No PC Code for EU which is degradate of ETU PC Code: 600016

Date Evaluation Completed: 02-13-2015

CITATION: Palmer, S.J., T.Z. Kendall, and H.O. Krueger 2001. Ethylene Urea: A 96-Hour Toxicity Test with the Freshwater Alga (*Selenastrum capricornutum*). Unpublished study performed by Wildlife International, Ltd.,Easton, Maryland. Laboratory Project Identification No.299A-116. Study submitted by EBDC/ETU Task Force, Washington, D.C. Experimental start date September 29, 2000 and experimental termination date October 3, 2000. The final report issued January 29, 2001.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of *Selenastrum capricornutum* were exposed to Ethylene urea (Degradate of ETU) under static conditions. The nominal test concentrations were 7.5, 15, 30, 60, and 120 ppm a.i., compared to a negative control. The mean measured concentrations were <4.00 (<LOQ, negative control), 7.5, 15, 29, 58, and 119 ppm a.i.

By 96 hours, the cell density percent inhibitions were 1.5, -3.3, -1.3, 0.076, and -5.3% for the mean-measured 7.5, 15, 29, 58, and 119 ppm a.i. treatment groups, respectively, compared to the negative control. Cell densities were not significantly reduced at any treatment level tested (p >0.05, William's). The cell density EC_{50} was >119 ppm a.i. and the NOEC was 119 ppm a.i.

The percent inhibitions for 0-96 hour biomass (mean area under the growth curve) were 1.8, -2.4, -1.8, 3.1, and -5.5% in the mean-measured 7.5, 15, 29, 58, and 119 ppm a.i. treatment groups, respectively, compared to the negative control. Biomass was not significantly reduced at any treatment level tested (p >0.05, William's). The biomass EC_{50} was >119 ppm a.i. and the NOEC was 119 ppm a.i.

The percent inhibitions for 0-96 hour growth rates were 0.27, -0.57, -0.23, 0.04, and -0.93% in the mean-measured 7.5, 15, 29, 58, and 119 ppm a.i. treatment groups, respectively, compared to the negative control. Growth rates were not significantly reduced at any treatment level tested (p >0.05, William's). The growth rate EC_{50} was >119 ppm a.i. and the NOEC was 119 ppm a.i.

This toxicity study is scientifically sound and satisfies the U.S. EPA Guideline Subdivision J, 850.4400 (previously FIFRA 123-2) for an aquatic nonvascular plant study with *Selenastrum capricornutum* [§123-2]. This study is classified as ACCEPTABLE.

Results Synopsis

Test Organism: *Selenastrum capricornutum*
Test Type: Static

Cell Density:
NOEC: 119 ppm a.i.
LOEC: >119 ppm a.i.
EC_{05}: >119 ppm a.i. 95% C.I.: N/A
EC_{50}/IC_{50} : >119 ppm a.i. 95% C.I.: N/A
Slope: N/A

Biomass (area under the growth curve):
NOEC: 119 ppm a.i.
LOEC: >119 ppm a.i.
EC_{05}: >119 ppm a.i. 95% C.I.: N/A
EC_{50}/IC_{50} : >119 ppm a.i. 95% C.I.: N/A
Slope: N/A

Growth Rates:
NOEC: 119 ppm a.i.
LOEC: >119 ppm a.i.
EC_{05}: >119 ppm a.i. 95% C.I.: N/A

EC_{50}/IC_{50} : >119 ppm a.i. 95% C.I.: N/A
Slope: N/A
Endpoint(s) Affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the U.S. EPA Series 850 - Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.5400: *Algal Toxicity Test, Tiers I and II*; OECD Guideline for Testing of Chemicals 201: *Alga, Growth Inhibition Test*; and Official Journal of the European Communities No. L383, Method C.3.: *Algal Inhibition Test*. The following deviations from U.S. EPA Guideline 123-2 are noted:

1. The pretest health of the test organism and the actual strain of *Selenastrum capricornutum* used in the definitive exposure were not reported.

2. The TOC and particulate matter and chlorine concentrations were not assessed in the periodic screening of the laboratory well water.

These deviations do not affect the acceptability or the validity of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. The study followed the U.S. EPA (40 CFR, Part 160 and 792, 1989) Good Laboratory Practice ; OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984.

A. MATERIALS:

1. Test Material Ethylene urea (EU), Degradate of Ethylenethiourea (ETU)

Description: Off-white crystalline solid

Lot No./Batch No. : 01743-141

Purity: 90.8%

Stability of Compound
Under Test Conditions: The measured concentrations of EU were 96.5-99.4% of nominal at hour 0, 96.7-101% of nominal at 72-hours, and 98.2-99.7% of nominal at 96-hours. Measured concentrations from the abiotic replicate were 99.7 and 101% of nominal at 72- and 96-hours, respectively.

(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Storage conditions of test chemicals: The test substance was stored under ambient conditions.

2. Test organism:

Name: *Selenastrum capricornutum*

EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. capricorntum, and a freshwater diatom is tested
OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported

Strain: Not reported
Source: Current in-house laboratory cultures. Originally obtained from University of Toronto Culture Collection.
Age of inoculum: Growing in culture medium for at least two weeks prior to test initiation.
Method of cultivation: Freshwater algal medium prepared with reagent-grade chemicals and NANOpure® water.

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: The definitive nominal test concentrations were selected in consultation with the Sponsor and were based on the results of a range-finding test, however, the results were not reported..

b) Definitive Study: The nominal concentrations selected were adjusted to 100% based on the reported test substance purity of 90.8%. All test concentrations were reported as mg test substance (t.s.)/L. The nominal test concentrations were 7.5, 15, 30, 60, and 120 mg t.s./L and were assumed to be equivalent to 7.5, 15, 30, 60, and 120 ppm a.i. by the Reviewer.

Table 1 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: culturing media and conditions: (same as test or not) health: (any toxicity observed)	At least two weeks. Freshwater algal medium prepared with reagent-grade chemicals and NANOpure® water; same as test. Not reported	Actively growing in culture medium for at least two weeks prior to test initiation. Algal medium was prepared according to ASTM Standard Guide 1218-90E. *EPA recommends two week acclimation period.* *OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.*
Test system static/static renewal: renewal rate for static renewal:	Static	
Incubation facility	Environmental chamber	
Duration of the test	96 hours	*EPA requires: 96 - 120 hours* *OECD: 72 hours*
Test vessel material: (glass/polystyrene) size: fill volume:	Glass Erlenmeyer flasks plugged with foam stoppers. 250 mL 100 mL	*OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity **(for marine algae):**	 Freshwater Algal Medium 7.3 7.7-8.7 disodium EDTA $NaHCO_3$	The pH was 7.5 in the abiotic replicate at test termination. *OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.*

Parameter	Details	Remarks *Criteria*
	N/A	*EPA recommends 20X-AAP medium.*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Yes	
Dilution water source: type: pH: salinity **(for marine algae):** water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Laboratory well water Sterilized by filtration medium was 7.5 ± 0.1 N/A Sterilized prior to use Not reported Not reported <LOD <LOD Not reported	Dilution water was sterilized and purified using a NANOpure® filtration system (0.22µm). *EPA pH: Skeletonema costatum= ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solution	
Aeration or agitation	Agitation, 100 rpm	Test vessel were indiscriminately positioned on mechanical shakers. *EPA recommends agitation only for Selenastrum at 100 cycles per min and Skeletonema at ~60 cycles per min. Aeration is not recommended.*
Initial cells density	Approximately 10,000 cells/mL	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Selenastrum capricornutum, cell counts on day 2 are not required.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricornutum and S. subspicatus. When other species are used the biomass should be comparable.*

Parameter	Details	Remarks *Criteria*
Number of replicates control: solvent control: treated ones:	 3 (biological) N/A 3 (biological)	One additional analytical replicate was maintained in each control and treatment group for analytical verification at 72-hours. Two additional abiotic replicates were maintained at the highest level tested for analytical verification at 72 and 95 hours. *EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicate.* *OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test cultures should be included in the test.*
Test concentrations nominal: measured:	 0 (negative control), 7.5, 15, 30, 60, and 120 ppm a.i. <4.00 (<LOQ, negative control), 7.5, 15, 29, 58, 119, and 121 ppm a.i.	The nominal and mean-measured treatment concentrations were reported in terms of mg test substance/L, the reviewer consider this unit to be synonymous to ppm a.i. for reporting purposes within this DER regardless of the fact that the test material is a degradate. *EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth*

Parameter	Details	Remarks *Criteria*
		completely.
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	HPLC; @ 0, 72, and 96 hours	QC matrix fortifications of 7.00, 30.0, and 125 ppm a.i. measured concurrently with the test samples had recoveries of 99.5-104% of nominal. The LOQ for the HPLC analytical method was 4.00 ppm a.i..
Test conditions temperature: photoperiod: light intensity and quality:	 22.4-23.6°C Continuous 3880-4690 lux	*EPA temperature: Skeletonema: 20℃, Others: 24-25℃; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* *OECD recommended the temperature in the range of 21 to25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*
Reference chemical {if used)	N/A	
Other parameters, if any	None	

2. Observations

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell densities, growth rate, and biomass (area under the growth curve).	*EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*

Parameters	Details	Remarks/Criteria
Measurement technique for cell density and other end points	Electronic particle counter (Coulter Electronics, Inc.), verified and initially calibrated using a hemacytometer and a microscope	*EPA recommends the measurement technique of cell counts or chlorophyll a* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).*
Observation intervals	Every 24 hours	*EPA and OECD: every 24 hours.*
Other observations, if any	None	
Indicate whether there was exponential growth in the control	Yes, dilution water control group cell densities at test termination were 289X greater, than the dilution water group cell densities at test initiation.	2,889,469 cells/ml at 96-hours and ~10,000 cells/ml at test initiation. *EPA requires control cell count at termination to be ≥2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Yes	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

By 96 hours, the cell density percent inhibitions were 1.5, -3.3, -1.3, 0.076, and -5.3% for the mean-measured 7.5, 15, 29, 58, and 119 ppm a.i. treatment groups, respectively, compared to the negative control. The percent inhibitions for 0-96 hour biomass (mean area under the growth curve) were 1.8, -2.4, -1.8, 3.1, and -5.5% in the mean-measured 7.5, 15, 29, 58, and 119 ppm a.i. treatment groups, respectively, compared to the negative control. The percent inhibitions for 0-96 hour growth rates were 0.27, -0.57, -0.23, 0.04, and -0.93% in the mean-measured 7.5, 15, 29, 58, and 119 ppm a.i. treatment groups, respectively, compared to the negative control. Cell densities, biomass, and growth rates were not significantly reduced at any treatment level compared to the negative control ($p>0.05$, Dunnett's test) by 96-hours.

Table 3: Effect of EU (Degradate of ETU) on Freshwater Green Algae (*Selenastrum capricornutum*)

Treatment Mean Measured and Nominal Concentrations [a] (ppm a.i.)	Initial Cell Density (cells/mL)	Mean Cell Density (cells/mL) at		
		24 Hours	96 Hours	
			Cell Count	% Inhibition
Dilution water control	10,000	39,477	2,889,469	--
7.5 (7.5)	10,000	35,868	2,845,920	1.5
15 (15)	10,000	34,485	2,984,622	-3.3
29 (30)	10,000	36,382	2,927,294	-1.3
58 (60)	10,000	34,420	2,887,264	0.076
119 (120)	10,000	35,411	3,043,906	-5.3
Reference chemical (if used)	N/A	N/A	N/A	N/A

[a] The nominal test concentrations are presented in parentheses.

Table 4: Effect of EU (Degradate of ETU) on Freshwater Green Algae (*Selenastrum capricornutum*)

Mean Measured and Nominal Treatment Concentrations [a] (ppm a.i.)	Initial Cell Density (cells/mL)	Mean Growth Rate (cells/ml/hour)	% Inhibition (Mean Growth Rate)	Mean Area Under Growth Curve	% Inhibition (Mean Area Under Growth Curve)
Dilution water control	10,000	0.0590	--	58,327,096	--
7.5 (7.5)	10,000	0.0589	0.27	57,279,132	1.8
15 (15)	10,000	0.0594	-0.57	59,700,896	-2.4
29 (30)	10,000	0.0592	-0.23	59,360,764	-1.8
58 (60)	10,000	0.0590	0.04	56,504,228	3.1
119 (120)	10,000	0.0596	-0.93	61,536,616	-5.5
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

[a] The nominal test concentrations are presented in parentheses.

Table 5: Statistical Endpoint Values.

Statistical Endpoint	Cell Density	Growth Rate	Biomass
NOEC or EC_{05} (ppm a.i.)	119	119	119
EC_{50} (ppm a.i.)	>119	>119	>119
IC_{50} or EC_{50} (ppm a.i.) (95% C.I.)	N/A	N/A	N/A
IC_{25}/EC_{25} (ppm a.i.) (and 95% C.I.)	ND	ND	ND
Reference chemical, if used NOAEC IC_{50}/EC_{50}	N/A	N/A	N/A

ND = Not determined
N/A = Not applicable

B. REPORTED STATISTICS:

Statistical Method: Cell densities, biomass (areas under the growth curve), growth rates and percent inhibitions values were calculated using "The SAS System for Windows" (Release 6.12). After confirming normality and homogeneity of variances using Shapiro-Wilk's and Levene's tests, respectively, cell densities, biomass, and growth rates response data were statistically compared to the control group using Dunnett's test. EC_{50} values for the above endpoints were visually estimated due to the lack of a 50% reduction at any treatment level tested. The reported NOEC, LOEC and EC_{50} values were determined in terms of the mean measured test concentrations.

Cell Density:
NOEC: 119 ppm a.i.
LOEC: >119 ppm a.i.
EC_{05}: Not reported 95% C.I.: N/A
EC_{50}/IC_{50} : >119 ppm a.i. 95% C.I.: N/A
Slope: N/A

Biomass (area under the growth curve):
NOEC: 119 ppm a.i.
LOEC: >119 ppm a.i.
EC_{05}: Not reported 95% C.I.: N/A
EC_{50}/IC_{50} : >119 ppm a.i. 95% C.I.: N/A
Slope: N/A

Growth Rates:
NOEC: 119 ppm a.i.
LOEC: >119 ppm a.i.
EC_{05}: Not reported 95% C.I.: N/A
EC_{50}/IC_{50} : >119 ppm a.i. 95% C.I.: N/A Slope: N/A

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: After confirming normality and homogeneity of variances using, cell density, biomass, and growth rate treatment response data were statistically compared to the negative control group using ANOVA and William's multiple comparison test via TOXSTAT statistical software. The NOEC and LOEC for each endpoint was determined based on the results of the above statistical analyses. EC_{05} and EC_{50} values for the above endpoints were visually estimated due to the lack of a ≥5% inhibition at any treatment level tested. All toxicity values were determined in terms of the mean measured test concentrations.

Cell Density:
NOEC: 119 ppm a.i.
LOEC: >119 ppm a.i.
EC_{05}: >119 ppm a.i. 95% C.I.: N/A
EC_{50}/IC_{50} : >119 ppm a.i. 95% C.I.: N/A
Slope: N/A

Biomass (area under the growth curve):
NOEC: 119 ppm a.i.
LOEC: >119 ppm a.i.
EC_{05}: >119 ppm a.i. 95% C.I.: N/A
EC_{50}/IC_{50} : >119 ppm a.i. 95% C.I.: N/A
Slope: N/A

Growth Rates:
NOEC: 119 ppm a.i.
LOEC: >119 ppm a.i.
EC_{05}: >119 ppm a.i. 95% C.I.: N/A
EC_{50}/IC_{50} : >119 ppm a.i. 95% C.I.: N/A
Slope: N/A

Endpoint(s) Affected: None

D. STUDY DEFICIENCIES:

All deviations/deficiencies were considered minor and did not affect the acceptability or validity of this study.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to those of the study authors'.

F. CONCLUSIONS: This toxicity study is scientifically sound and satisfies the U.S. EPA Guideline Subdivision J, 850.4400 for an aquatic nonvascular plant study with *Selenastrum capricornutum*. This study is classified as ACCEPTABLE.

Cell Density:
NOEC: 119 ppm a.i.
LOEC: >119 ppm a.i.
EC_{05}: >119 ppm a.i. 95% C.I.: N/A
EC_{50}/IC_{50} : >119 ppm a.i. 95% C.I.: N/A
Slope: N/A

Biomass (area under the growth curve):
NOEC: 119 ppm a.i.
LOEC: >119 ppm a.i.
EC_{05}: >119 ppm a.i. 95% C.I.: N/A
EC_{50}/IC_{50} : >119 ppm a.i. 95% C.I.: N/A
Slope: N/A

Growth Rates:
NOEC: 119 ppm a.i.
LOEC: >119 ppm a.i.
EC_{05}: >119 ppm a.i. 95% C.I.: N/A
EC_{50}/IC_{50} : >119 ppm a.i. 95% C.I.: N/A
Slope: N/A

Endpoint(s) Affected: None

III. REFERENCES:

U.S. EPA. 1996. Series 850 - Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.5400: *Algal Toxicity*, Tiers I and II.

OECD. 1984. OECD Guidelines for Testing of Chemicals, 201: *Alga, Growth Inhibition Test*.

Official Journal of the European Communities. 1992. No. L383. Method C.3. Algal Inhibition Test.

ASTM Standard Guide 1218-90E. 1990. *Standard Guide for Conducting Static 96-Hour Toxicity Test with Microalgae*. American Society for Testing and Materials. Philadelphia, PA.

The SAS System for Windows. 1996. Release 6.12, TS Level 0020. SAS Institute Inc., Cary, North Carolina.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Cell Densities (cells/ml; 96-hours)
File: 2904cd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	79510943196.000	15902188639.203	0.813
Within (Error)	12	234665691904.000	19555474325.344	
Total	17	314176635100.000		

Critical F value = 3.11 (0.05,5,12)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

Cell Densities (cells/ml; 96-hours)
File: 2904cd Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	2889469.333	2889469.333		
2	7.5	2845919.667	2845919.667	0.381	
3	15	2984622.000	2984622.000	-0.833	
4	29	2927294.333	2927294.333	-0.331	
5	58	2887264.333	2887264.333	0.019	
6	119	3043906.000	3043906.000	-1.353	

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

Cell Densities (cells/ml; 96-hours)
File: 2904cd Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	3			
2	7.5	3	285449.020	9.9	43549.667
3	15	3	285449.020	9.9	-95152.667
4	29	3	285449.020	9.9	-37825.000
5	58	3	285449.020	9.9	2205.000
6	119	3	285449.020	9.9	-154436.667

Cell Densities (cells/ml; 96-hours)
File: 2904cd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	3	2889469.333	2889469.333	2867694.500
2	7.5	3	2845919.667	2845919.667	2867694.500
3	15	3	2984622.000	2984622.000	2933060.222
4	29	3	2927294.333	2927294.333	2933060.222
5	58	3	2887264.333	2887264.333	2933060.222
6	**119**	**3**	**3043906.000**	**3043906.000**	**3043906.000**

Cell Densities (cells/ml; 96-hours)
File: 2904cd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	2867694.500				
7.5	2867694.500	0.191		1.78	k= 1, v=12
15	2933060.222	0.382		1.87	k= 2, v=12
29	2933060.222	0.382		1.90	k= 3, v=12
58	2933060.222	0.382		1.92	k= 4, v=12
119	**3043906.000**	**1.353**		**1.93**	**k= 5, v=12**

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s = 139840.889
Note: df used for table values are approximate when v > 20.

Biomass (0-96 hours)
File: 2904bd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	49262986232320.000	9852597246464.000	0.981
Within (Error)	12	120491534188544.000	10040961182384.000	
Total	17	169754520420864.000		

Critical F value = 3.11 (0.05,5,12)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

Biomass (0-96 hours)
File: 2904bd Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	58327096.000	58327096.000		
2	7.5	57279132.000	57279132.000	0.405	
3	15	59700896.000	59700896.000	-0.531	
4	29	9360764.000	59360764.000	-0.400	
5	58	56504228.000	56504228.000	0.705	
6	119	61536616.000	61536616.000	-1.241	

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

Biomass (0-96 hours)
File: 2904bd Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	3			
2	7.5	3	6468178.898	11.1	1047964.000
3	15	3	6468178.898	11.1	-1373800.000
4	29	3	6468178.898	11.1	-1033668.000
5	58	3	6468178.898	11.1	1822868.000
6	119	3	6468178.898	11.1	-3209520.000

Biomass (0-96 hours)
File: 2904bd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control		358327096.000	58327096.000	57803114.000
2	7.5		357279132.000	57279132.000	57803114.000
3	15		359700896.000	59700896.000	58521962.667

4	29	359360764.000	59360764.000	58521962.667
5	58	356504228.000	56504228.000	58521962.667
6	**119**	**361536616.000**	**61536616.000**	**61536616.000**

Biomass (0-96 hours)
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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	57803114.000				
7.5	57803114.000	0.203		1.78	k= 1, v=12
15	58521962.667	0.075		1.87	k= 2, v=12
29	58521962.667	0.075		1.90	k= 3, v=12
58	58521962.667	0.075		1.92	k= 4, v=12
119	**61536616.000**	**1.241**		**1.93**	**k= 5, v**=12

s = 3168747.573
Note: df used for table values are approximate when v > 20.

Growth Rates (cell/ml/hour; 0-96 hours)
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	99.167	19.833	0.811
Within (Error)	12	293.333	24.444	
Total	17	392.500		

Critical F value = 3.11 (0.05,5,12)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

Growth Rates (cell/ml/hour; 0-96 hours)
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DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG

1	neg control	590.000	590.000	
2	7.5	588.667	588.667	0.330
3	15	593.667	593.667	-0.908
4	29	591.667	591.667	-0.413
5	58	589.667	589.667	0.083
6	119	595.333	595.333	-1.321

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

Growth Rates (cell/ml/hour; 0-96 hours)
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DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	3			
2	7.5	3	10.092	1.7	1.333
3	15	3	10.092	1.7	-3.667
4	29	3	10.092	1.7	-1.667
5	58	3	10.092	1.7	0.333
6	119	3	10.092	1.7	-5.333

Growth Rates (cell/ml/hour; 0-96 hours)
File: 2904gd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	3	590.000	590.000	589.333
2	7.5	3	588.667	588.667	589.333
3	15	3	593.667	593.667	591.667
4	29	3	591.667	591.667	591.667
5	58	3	589.667	589.667	591.667
6	**119**	**3**	**595.333**	**595.333**	**595.333**

Growth Rates (cell/ml/hour; 0-96 hours)
File: 2904gd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM

neg control	589.333				
7.5	589.333	0.165		1.78	k= 1, v=12
15	591.667	0.413		1.87	k= 2, v=12
29	591.667	0.413		1.90	k= 3, v=12
58	591.667	0.413		1.92	k= 4, v=12
119	**595.333**	**1.321**		**1.93**	**k= 5, v=12**

s = 4.944
Note: df used for table values are approximate when v > 20.